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VITELLINS AND VITELLOGENINS OF THE TERRESTRIAL ISOPOD, *ARMADILLIDIUM VULGARE*

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Abstract

Four forms of vitellogenin (Vg·1–Vg·4) in the hemolymph and four forms of vitellin (Vn·1–Vn·4) in the oocytes of reproductive females of *Armadillidium vulgare* were detected by polyacrylamide gel electrophoresis (PAGE) at stage D of the molting cycle. All vitellogenins decreased and were not detected in the hemolymph at stage E. At stage E, Vn·1–Vn·3 disappeared; Vn·4 was the major vitellin component in mature oocytes. The electrophoretic patterns of vitellogenin and vitellin revealed changes correlated with the molting cycle and oocyte growth.

Vitellogenins were electrophoretically identical to vitellins. Rabbit anti-Vn·4 antibody cross-reacted with vitellogenins. Using Slater's method, the four forms of vitellin were glycolipoproteins, with molecular weights of Vn·1–700,000, Vn·2–620,000, Vn·3–540,000, and Vn·4–470,000. In SDS-PAGE, vitellin Vn·4 yielded three main polypeptide components with molecular weights of 80,000, 99,000, and 127,000. The polypeptide compositions among vitellins (Vn·1–Vn·4) were similar.

Introduction

The correlation between the molting cycle and secondary vitellogenesis was described in the amphipod *Orchestia gammarellus* (Meusy and Charniaux-Cotton, 1984). In the isopod *Porcellio dilatatus*, the fat body (subepidermal adipose tissue) is the site of vitellogenin synthesis (Picaud and Souty, 1980). The cauterization of Y-organs (Souty *et al.*, 1982; Picaud, 1983) and ovariectomy (Picaud and Souty, 1981) of *P. dilatatus* lowered the rate of vitellogenin release into the hemolymph. However, no study elucidates the hormonal mechanisms which regulate the vitellogenin synthesis in Crustacea.

I am investigating the hormonal control of reproduction—especially hormonal regulation of vitellogenin synthesis—in *Armadillidium vulgare*. Previous work on *A. vulgare* indicated that rapid oocyte growth occurs at stage D of the molting cycle. Oocytes did not continue to grow in Y-organ ablated females (Suzuki, 1986). Y-organ (molting hormone) was needed for oocyte growth. However, it is not yet known whether vitellogenin synthesis is induced by molting hormone in *A. vulgare*.

The present study identified female-specific proteins (vitellins and vitellogenins) of *A. vulgare* by electrophoresis. The data will contribute to the understanding of vitellogenin synthesis and its hormonal (molting hormone) regulation. This paper identifies four forms of vitellogenins and vitellins, and describes their characteristics in *A. vulgare*. Three of the forms were described by Picaud (1983).

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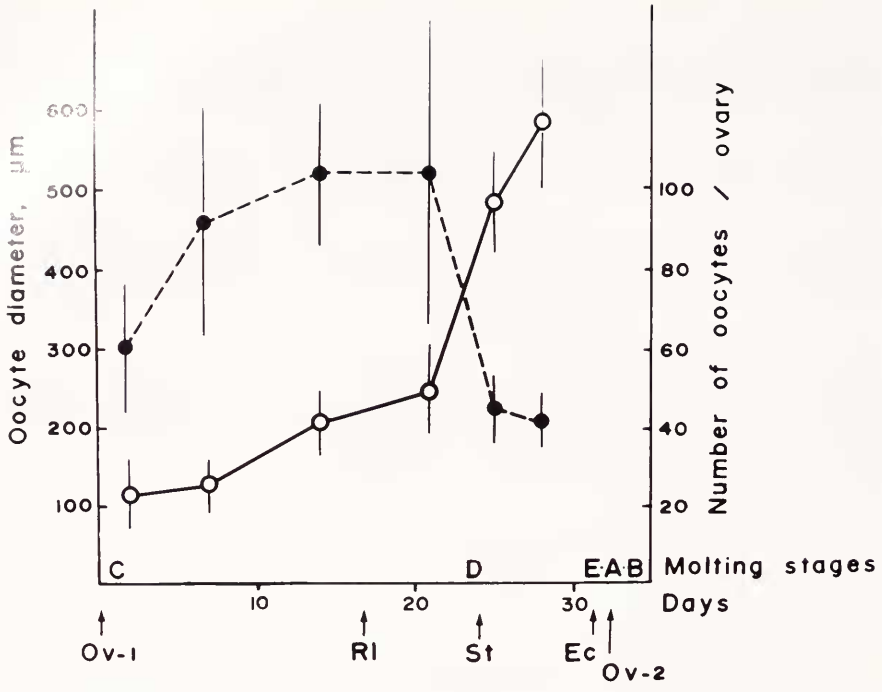

FIGURE 1. Oocyte growth during the molting cycle following first oviposition in *Armadillidium vulgare*. — ○ —, diameter of oocyte; -- ● --, number of oocytes; Ov-1, first oviposition; Ov-2, second oviposition; RI, release of larva; St, sternoliths; Ec, ecdysis. Ventrical lines around the points show standard errors.

MATERIALS AND METHODS

Animals

Armadillidium vulgare was collected near Yokohama in early March 1986. Several females (10–12 mm body length) were kept in a petri dish with moistened soil. Two adult males were added to the dish for a normal reproductive cycle. They were maintained at 25 ± 2°C in natural daylight, and fed decayed leaves and rat chows.

To observe oocyte growth, fresh ovaries were examined microscopically.

Preparation of hemolymph and ovarian homogenate to electrophoresis

Hemolymph samples were collected using a capillary tube placed in a hole in the tergite of the seventh thoracic segment. It was diluted 1/10 with Tris-HCl buffer (20 m*M*, pH 7.6 containing 130 m*M* NaCl and 5 m*M* EDTA). Dissected ovaries were rinsed and homogenated in 300 μl Tris-HCl buffer. Ovarian homogenate was passed through prefilter (milipore, AP) to remove lipids, centrifuged at 20,000 × *g* for 15 min at 4°C, and the supernatant was collected as a sample of ovarian homogenate.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was performed using 5% acrylamide monomer (Davis, 1964). Polyacrylamide gel electrophoresis in sodium dodecyl sul-

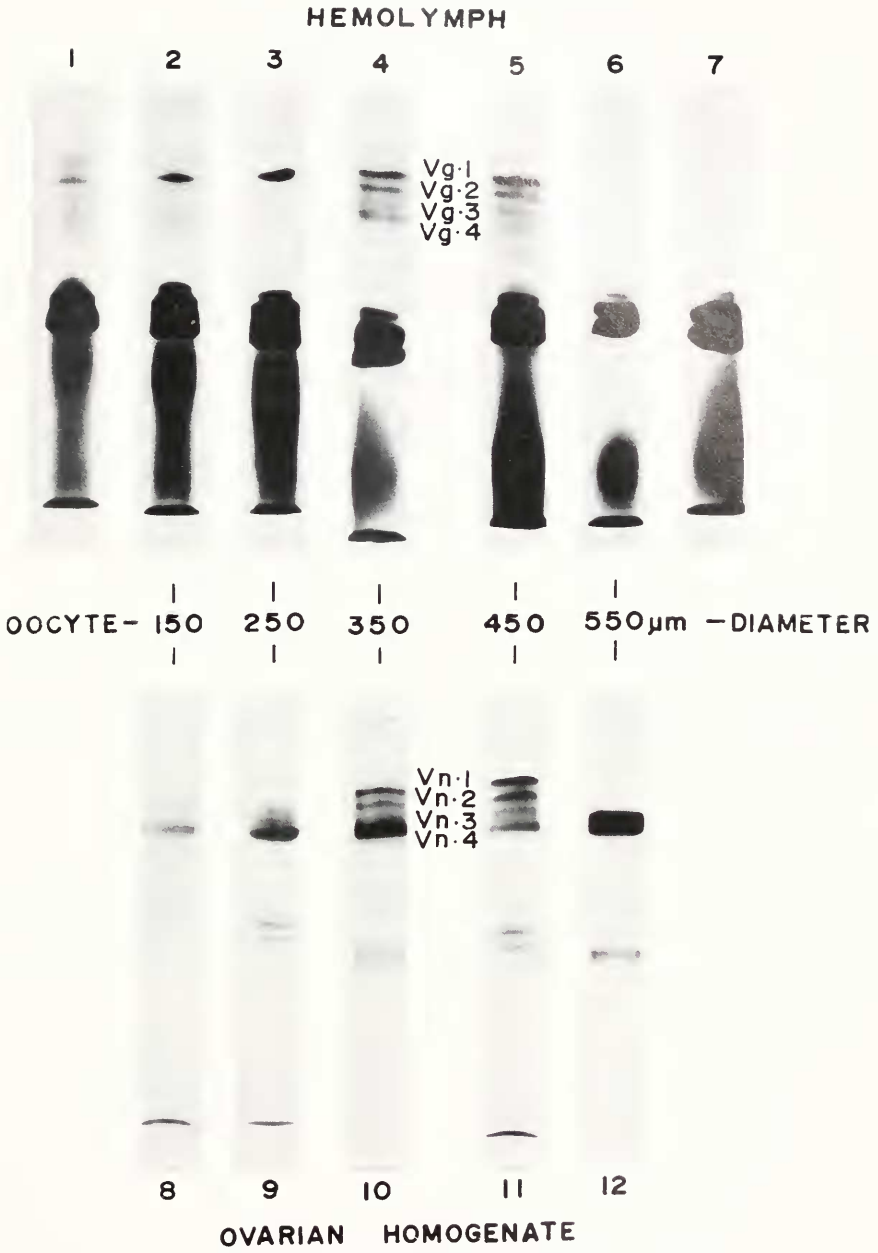

FIGURE 2. Electrophoretic pattern of the hemolymph and the ovarian homogenate on 5% PAGE. 1, hibernating adult females; 2–6 and 8–12, vitellogenic females; 7, adult male; Vg·1–Vg·4, vitellogenin; Vn·1–Vn·4, vitellin.

fate (SDS-PAGE) was also performed (Laemmli, 1970) using 10% acrylamide. Pore-limited electrophoresis was carried out on 5–25% acrylamide gradient slab gels (PAGGE) (Slater, 1969) using Davis's buffer system.

FIGURE 3. PAGE of the ovarian homogenate (350–450 μm in oocyte diameter) stained for protein with Coomassie blue (1), for glycoprotein with periodic acid/Schiff reagent (2), and for lipoprotein with Sudan black B (3).

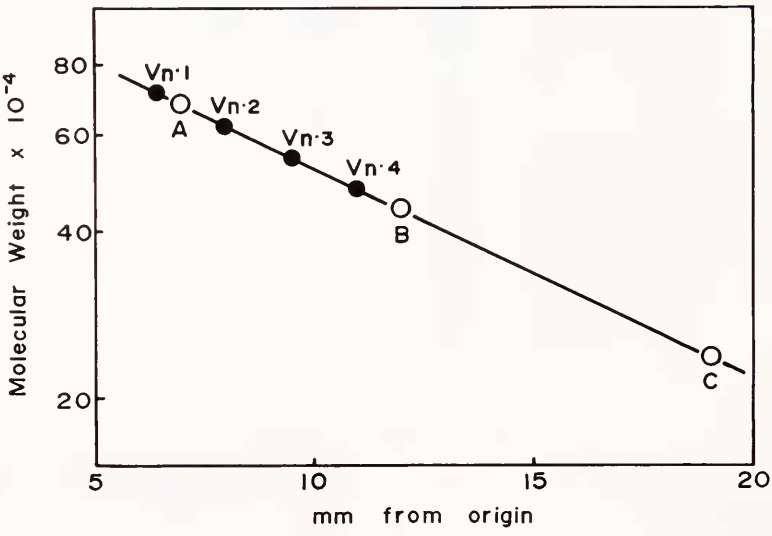

FIGURE 4. Molecular weight determination of vitellins by pore-limited electrophoresis (5–25% PAGGE) of ovarian homogenate (350–450 μm). Molecular weights were calculated from mobilities relative to each standard proteins (A–C, Pharmacia). A, thyroglobulin (669,000 M. W.); B, ferritin (440,000); C, catalase (232,000); Vn·1–700,000; Vn·2–620,000; Vn·3–540,000; Vn·4–470,000.

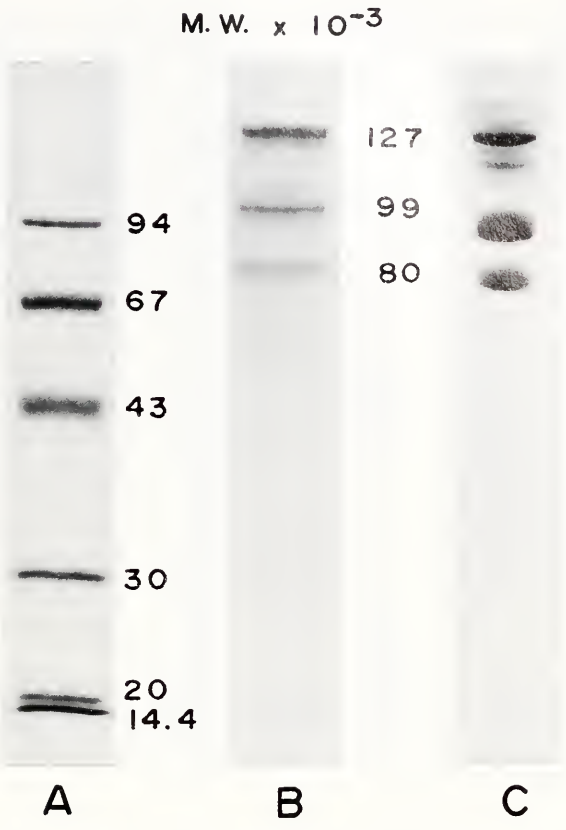

FIGURE 5. Molecular weight determination of Vn·4 polypeptide by SDS-PAGE of ovarian homogenate. A, slab gel of standard proteins (phosphorylase b-94,000; bovine serum albumine-67,000; ovalbumin-43,000; carbonic anhydrase-30,000; soybean trypsin inhibitor-20,000; α-lactalbumin-14,400, Pharmacia). B, slab gel of ovarian homogenate (150 μm in oocyte diameter and stage C). C, disc gel of ovarian homogenate (500 μm and stage E).

After electrophoresis, proteins were stained with Coomassie blue and glycoproteins were visualized using the periodic acid Schiff (PAS) method (Zacharius *et al.*, 1969). Lipoprotein samples were stained with Sudan black B (Sano, 1981) prior to electrophoresis.

Preparation of antibody and immunodiffusion test

VN·4 was used as antigen. Mature oocytes (200, 550 μm in diameter) were homogenized in Tris-HCl buffer and centrifuged. The resulting 1.5 ml containing about 1 mg/ml total proteins (Lowry *et al.*, 1951, with BSA as a standard) was applied to the top of a slab gel (PAGE, 3 mm thick, 10.5 × 11.5 cm, 30 mA for 2 h). After electrophoresis, both sides of the gel were stained with Coomassie blue to define the vitellin band. The Vn·4 band was then cut from the unstained portion of the slab gel and thoroughly homogenized with 1.5 ml Tris-HCl buffer. The gel homogenate (3 ml) was stored frozen at −80°C until use.

One milliliter of the homogenate (350 μg/ml proteins) and Freund's complete

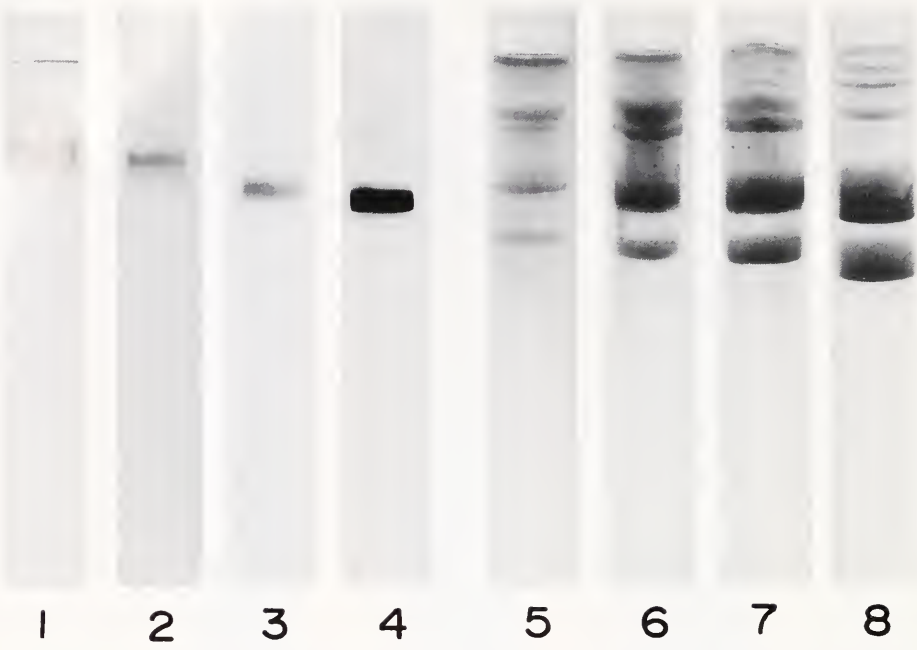

FIGURE 6. Polypeptide analysis of vitellin Vn·1–Vn·4. Ovarian homogenate (350–450 μm) was prestained with Sudan black B. After PAGE of homogenate, each vitellin band was cut out of the disc gels and eluted by homogenizing the gel bands in 20 m*M* Tris-HCl buffer. PAGE patterns of separated Vn·1 (1), Vn·2 (2), Vn·3 (3), and Vn·4 (4). Polypeptide analysis of each separated Vn·1 (5), Vn·2 (6), Vn·3 (7), and Vn·4 (8) on disc SDS-PAGE.

adjuvant (1 ml) were injected subcutaneously into the back of a male rabbit twice every 10 days. A booster injection was given once, on the 10th day after the second antigen injection. Five days later the rabbit was bled and an immunoglobulin (IgG) fraction was precipitated by ammonium sulfate (40% saturation). After dialysis against Tris-HCl buffer, this antiserum (anti-Vn·4 IgG) was stored frozen at −80°C.

Immunodiffusion tests were performed (Ouchterlony, 1949). Samples were tested on 1% agarose gel plates with 0.5 *M* Tris-HCl buffer. Both antigen and antibody were allowed to diffuse for one day at room temperature and examined for precipitin lines.

RESULTS

Changes of vitellin and vitellogenin during the molting cycle

Young oocytes were seen (Fig. 1) in the ovaries after oviposition. They gradually increased in size during stage C of the molting cycle. Rapid oocyte growth began soon after the appearance of the sternoliths, which were evident in stage D. Oocytes were about 550 μm at stage E, and ecdysis occurred in the posterior and then anterior region of the female's body before oviposition.

The electrophoretic pattern of vitellogenins and vitellins was investigated on disc PAGE. Diluted hemolymph (20 μl) and ovarian homogenate (30 μl) were subjected to electrophoresis at 2 mA per tube for 3 h. Hemolymph analysis revealed four forms of vitellogenin (Vg·1–Vg·4) present in the hemolymph of stage D females with sub-

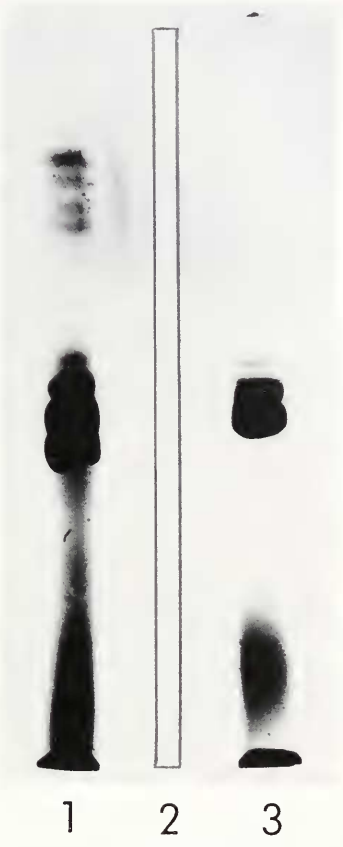

FIGURE 7. Identification of vitellogenin by immunoelectrophoresis. Hemolymph from reproductive female (1) and male (3) at stage D of the molting cycle were subjected to PAGE. Disc gels were then embedded in a layer of 1% agarose gel on a glass plate. The trough (2) of the agarose gel was cut and filled with anti-Vn·4 serum. After 24 h diffusion at room temperature, PAGE gels of (1 and 3) were removed and the precipitate line of immunochemical reaction was stained with Coomassie blue. The other PAGE gels of (1 and 3), stained with Coomassie blue, were placed on each original site of the agarose gel.

mature oocytes (350–450 μm) (Fig. 2·4, 5). These forms were not detected in females with mature oocytes (550 μm) at stage E of the molting cycle (Fig. 2·6). Vitellogenin was not detected either in stages A and B of the reproductive females or in the hemolymph of males (Fig. 2·7) throughout the molting cycle.

Analysis of ovarian homogenate revealed four forms of vitellin (Vn·1–Vn·4) in immature oocytes of stage D females (Fig. 2·10, 11). These forms of vitellin exhibited the same electrophoretic pattern as vitellogenin. At stage E, Vn·1–Vn·3 were not detected; Vn·4 was the major component of vitellin found in mature oocytes (Fig. 2·12). Vn·4 was also the primary vitellin in young oocytes of stage C females (Fig. 2·8, 9).

Characterization of vitellogenins and vitellins

The four bands of vitellin and vitellogenin had low electrophoretic mobility and were located closely together (Fig. 2·5, 11). Figure 3 shows that the vitellins were

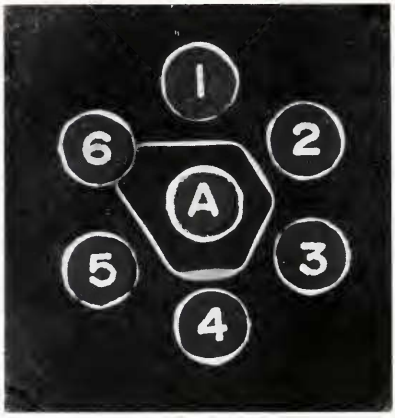

FIGURE 8. Ouchterlony agar diffusion analysis showing reaction between anti-Vn·4 serum (A) against ovarian homogenate (1 and 2) or hemolymph of females (3–6). 1 and 4, stage C; 2 and 5, stage D; 3, hibernating; 6, stage E.

glycolipoproteins, as stained with periodic acid/Schiff reagent and Sudan black B. The ovarian homogenate, prepared from immature oocytes (350–450 μm), was analyzed by the pore-limited method (PAGGE) using standard proteins to determine the molecular weight of the four vitellins (Fig. 4). They were estimated to be Vn·1–700,000 (700K), Vn·2–620,000 (620K), Vn·3–540,000 (540K), and Vn·4–470,000 (470K), respectively.

Figures 5 and 6 show the polypeptide composition of vitellin separated in SDS-PAGE. Vn·4, the major vitellin in stages C and E (Fig. 2·8, 12), yielded three main polypeptides with molecular weights estimated to be 80,000 (80K), 99,000 (99K), and 127,000 (127K) (Fig. 5). To compare the polypeptide compositions of Vn·1–Vn·4, each vitellin prestained with Sudan black B was isolated from immature oocytes using PAGE. After electrophoresis, the gel band of each vitellin was cut out and eluted in Tris-HCl buffer. Although the isolated vitellin overlapped with other vitellins (Fig. 6·1–4) each vitellin was analyzed on SDS-PAGE. Vn·1–Vn·4 were separated into several polypeptides; their main polypeptide compositions were very similar (Fig. 6·5–8). The lower polypeptides (MW 80K and 99K) were common through Vn·1–Vn·4 (Figs. 5, 6).

Immunochemical reactions of anti-Vn·4 serum against ovarian homogenate (vitellin) and diluted hemolymph (vitellogenin) were examined by double diffusion analysis in agarose gel. The four vitellogenins gave a single precipitate line when anti-Vn·4 serum was reacted with the hemolymph of stage D females. The precipitate line was absent when the serum was reacted with hemolymph of males (Fig. 7). Figure 8 presents the reactions between anti-Vn·4 serum and various stage hemolymph or ovarian homogenates. These results indicate that anti-Vn·4 antibody cross-reacted with vitellins and vitellogenins.

DISCUSSION

Four forms of vitellin and vitellogenin were identified electrophoretically from *Armadillidium vulgare*. Picaud (1983) found three forms from this species by electrophoresis. Vitellins are glycolipoproteins with higher molecular weights (700K–470K).

Vn·4, the smallest vitellin, contains three main polypeptides at MW 80K, 99K, and 127K. The polypeptide compositions of Vn·1–Vn·4 are quite similar. Vitellins of the isopods *Porcellio dilatatus* (315K), *Idotea balthica* (290K), and *Ligia oceanica* (320K) have similar molecular weights (Picaud, 1983); molecular weights of *A. vulgare* vitellins are higher. However, the main polypeptide compositions of Vn·4 from *A. vulgare* (80K, 99K, and 127K) have molecular weights similar to those of *P. dilatatus* (78K, 97K, and 180K) (Picaud, 1983).

The presence of a precursor (vitellogenin) of egg yolk protein (vitellin) shows that *A. vulgare* vitellin appears to be synthesized at an extra ovarian site (possibly the fat body) and then transported to the ovary through the hemolymph, as in *Porcellio dilatatus* (Picaud and Souty, 1980). However, Souty (1983) reported that the ovary of *P. dilatatus* can synthesize its proteinic yolk.

Changes of vitellin and vitellogenin were observed during the molting cycle and oocyte growth. All forms of vitellin and vitellogenin were observed at stage D and, except for Vn·4, they declined and disappeared during oocyte growth. The major peak of vitellogenin synthesis was observed at stage D of the isopod in *Idotea balthica* by Souty and Picaud (1981) and in *Porcellio dilatatus* by Picaud and Souty (1981). The question remains whether there is a correlation between the changes in the appearance of vitellin and/or vitellogenin and the Y-organ (molting hormone) in *A. vulgare*. In isopods, however, Charniaux-Cotton (1985) found that molting hormone is necessary for vitellogenin synthesis and is regulated by a feedback mechanism.

Since vitellin Vn·1–Vn·4 have common polypeptides and a common antigenic determinant, the smallest vitellin (Vn·4) may be accumulated throughout oocyte growth. Vn·1–Vn·3 seem to undergo a proteolytic processing in the oocytes. Analogous phenomena have been reported (Baert, 1985, 1986) for *Perenerís cultrifera* (polychaete). During oocyte growth, the higher molecular weight vitellins disappeared leaving the single form (the lowest vitellin) at immaturity. These results suggest that a progressive proteolytic cleavage of vitellin occurs in polychaetes. Vitellogenin processing has also been reported in amphipods (Junéra and Meusy, 1982) and in the locust (Chen *et al.*, 1978). The processing of vitellin and vitellogenin is presently unclear in *A. vulgare* and awaits further study.

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